Objective: To provide the clinicopathologic findings of a patient who developed the clinical characteristics of Best disease (typically considered a juvenile macular degeneration) at the age of 75 years after being documented to be ophthalmoscopically normal at the age of 51 years.

Design: A member of a large family with Best disease, possessing a Y227N mutation in the VMD2 gene (the gene responsible for the disease, which encodes the bestrophin protein), developed small vitelliform lesions in both eyes at the age of 75 years and later developed yellow fleck-like depositions at the level of the retinal pigment epithelium (RPE), which were also identified in fundus photographs of family members. The patient died at the age of 93 years, and the histological features of the macular lesion and peripheral flecks were examined.

Results: Histopathologically, the retinal outer nuclear layer was attenuated, particularly in the macula. This attenuation was frequently associated with normal RPE. A large area of photoreceptor degeneration was present in the central macula, with loss of the underlying RPE cells. Outside of this region, the RPE density was within normal limits. The peripheral flecks were clusters of basal laminar deposits and drusen. Bestrophin immunohistochemistry revealed labeling along both the basolateral and apical membranes of the RPE.

Conclusions: Findings characteristic of Best disease may not manifest in a molecularly affected individual until late in life. Mutations in bestrophin appear to lead to extracellular deposit formation outside the macula in some families. The distribution of bestrophin in the RPE suggests that the protein may be mistargeted in those with Best disease who have the Y227N mutation, and that this may be a cause of the associated RPE and photoreceptor dysfunction.

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METHODS

REPORT OF A CASE

This patient belongs to a large family with Best disease that has been previously described. He was carefully examined at the age of 51 years, when his visual acuity was 20/20 OU and a completely normal fundus in each eye was documented photographically (Figure 1A and B).
(this was patient III:8 in the original report). He was seen intermittently during the next 24 years. At age 75 years, he returned with a visual acuity of 20/25 OU and small vitelliform lesions approximately one-third disc area in size centered on fixation (Figure 1C and D). Electro-oculographic recordings were reduced in both eyes: 1.2 OD and 1.1 OS. At the age of 77 years, he had eccentric vitelliform lesions in both eyes. He was followed up regularly until the age of 88 years. At that time, his visual acuity was 20/30 OD and 20/70 OS. The central vitelliform lesions had evolved into small knobs of gliotic tissue (Figure 2A and B). The eccentric vitelliform lesions had resolved, leaving patches of RPE atrophy. Multiple crumblike yellow flecks were observed in the midperiphery of both eyes at the level of the RPE. Two other members of his extended family exhibited similar midperipheral flecks later in life (Figure 2C and D). All affected members of this family harbor a heterozygous Y227N sequence variation in the bestrophin gene.8 Optical coherence tomography of the patient’s sister revealed an optically clear lesion at the foveal center (Figure 2E). The patient died at the age of 93 years owing to complications of stomach cancer.

HISTOPATHOLOGIC PROCEDURES

Both eyes were received and processed at approximately 6½ hours after death. Anterior segments were removed, and the eyes were photographed and dissected into central, temporal, nasal, inferior, and superior regions. Additional samples and cells from these eyes were collected and preserved for future studies. Both maculae were fixed in 4% paraformaldehyde in 100mM sodium cacodylate (pH 7.4) for 2 hours. The right macula was embedded in paraffin according to standard procedures. A superior wedge was collected from the right eye; this section contained some of the flecks observed ophthalmoscopically. The neural retina and the RPE-choroid-sclera from the left macula were embedded in acrylamide, as described previously.15 In addition, a 2-mm trephine punch was collected from the left macula, centered approximately 3 mm from the foveal center. This punch was incubated in diamidino-phenol-indole (Molecular Probes, Eugene, Ore), and was mounted with the RPE facing up for quantitation of the macular RPE. Diamidino-phenol-indole–stained nuclei were counted from 8 fields photographed with a ×40 objective. A sagittal wedge of the right eye was embedded into optimal cutting temperature compound without prior fixation.

Bestrophin immunohistochemistry was performed using a monoclonal antibody (NB 300-164; Novus Biologicals, Littleton, Colo) and secondary antibodies (Alexa 488 conjugated) directed against mouse IgG (Molecular Probes). Immunohistochemistry was performed as described previously.16,17 Immunolabeling of unfixed sections was detected on an MRC 1024 confocal microscope (BioRad, Hercules, Calif) using 488-nm
excitation. Autofluorescence of RPE lipofuscin was simultaneously detected at 568 nm, and nuclear counterstaining with TO-PRO-3 (Molecular Probes) was observed on excitation at 647 nm. Other probes used included an alkaline phosphatase substrate kit (to detect vessels) (BCIP/NBT) and biotinylated peanut agglutinin (both from Vector Laboratories, Burlingame, Calif), antibodies directed against rhodopsin (clone RetP1; Lab Vision, Fremont, Calif), fibrinogen (DAKO, Carpinteria, Calif), and glial fibrillar acidic protein (Sigma Chemical, St Louis, Mo). For confocal microscopy, sections with bestro-

Figure 2. Composite fundus images of both eyes of the patient at the age of 88 years (A and B), the eyes of family members (C and D), and optical coherence tomographic findings of the patient’s sister (E). By the age of 88 years, the central vitelliform lesions have developed gliotic scars. Multiple crumblike flecks are noted at the level of the retinal pigment epithelium (RPE) in both eyes (A and B). Part C is a photographic montage of the right eye of the patient’s sister, aged 74 years. There is an eccentric area of atrophy and lipofuscin material in the macula. Small crumblike flecks are noted at the level of the RPE in the midperiphery of this eye and the left eye (not shown). Her visual acuity in this eye was 20/40. Part D is a photographic montage of the right eye of the patient’s son at the age of 54 years. There is midperipheral lipofuscin deposition that is slightly different than observed in his father and his aunt. The other eye demonstrates a similar pattern (not shown). The visual acuity in this eye was 20/40. Part E shows the optical coherence tomographic findings of the left eye of the patient’s sister, obtained through the vitelliform lesion from nasal (left) to temporal (right). There is an optically clear central lesion and reflective RPE terminating at the nasal aspect of the lesion (between the arrows).
phin immunolabeling were compared with unfixed cryostat sections from 2 unaffected human donors. All participants in this study consented to participate, and institutional review board approvals were obtained from The University of Iowa Human Subjects' Committee.

RESULTS

A 4-mm trephine punch of the left macula was collected for light microscopy. Removal of the neural retina revealed a small area of RPE degeneration that included the fovea (Figure 3A and B). Histological observation of the left macula revealed outer nuclear layer attenuation and a region of severe photoreceptor degeneration resembling a scar, with eosinophilic material in the space normally occupied by the outer nuclear layer and inner and outer segments (Figure 3C). This material did not exhibit immunoreactivity for glial fibrillar acidic protein, but did react with antibodies directed against fibrinogen (Figure 3D). Labeling with anti–glial fibrillar acidic protein was unremarkable throughout the section, although glial fibrillar acidic protein–positive glial cells were observed outside the scar and at the interface between the scar and Bruch’s membrane. No evidence of alkaline phosphatase–positive vessels was detected within this material. The RPE degeneration occurred abruptly (Figure 3E),

Figure 3. Histological features of the left eye of the patient. The appearance of the macula of the left eye before (A) and after (B) removal of the neural retina is shown; there is a central region of retinal pigment epithelial (RPE) atrophy. In the center of this region of RPE atrophy, the outer nuclear layer is severely disrupted. In acrylamide-embedded cryostat sections, eosinophilic material occupies most of the outer nuclear layer, with some structural changes resembling a rosette (asterisk) (C). The material depicted in C shows immunoreactivity for fibrinogen (D). This area of retinal degeneration corresponds to the region of RPE atrophy depicted (E and F). The underlying area of RPE degeneration begins abruptly, with an intact Bruch’s membrane throughout (arrows) (E), and shows preservation of viable choriocapillaris as assessed by alkaline phosphatase enzyme activity (arrowheads) (F). In C and E, hematoxylin-eosin was used; D, antifibrinogen; and F, an alkaline phosphatase substrate kit (BCIP/NBT). Brightness and contrast were adjusted on light micrographs. GCL indicates ganglion cell layer; INL, inner nuclear layer.
with an intact Bruch’s membrane in the region of RPE atrophy. Capillaries directly below the atrophic RPE generally retained alkaline phosphatase activity (Figure 3F). The inner retina was intact in these regions.

Paraffin sections spanning the distance from the temporal pars plicata to the optic nerve were collected from the right eye and stained with hematoxylin-eosin. The most pronounced feature on histological examination was a remarkable degree of outer nuclear layer attenuation, most prominent in the macula (Figure 4A). Only 1 to 2 rows of outer nuclear layer nuclei were present, compared with approximately 4 to 7 layers in a healthy macula.
of this age, and the outer segments associated with these nuclei were positive for a rhodopsin monoclonal antibody. The RPE was largely intact and was of normal thickness in these areas. Bruch’s membrane was continuous and unfragmented throughout the eye. Much scarring was observed immediately temporal to the optic nerve head (Figure 4B).

The RPE density was assessed from photographs taken from a 2-mm punch centered approximately 3 mm temporal to the foveal center and stained with diaminophenol-indole. The measured mean RPE density was 5231 cells per square millimeter, with an SD of 713 cells per square millimeter. This value is consistent with RPE density measurements from other studies, and is higher than the mean for a donor eye of this age.

To determine the nature of the flecks observed on fundus examination, sections were taken through these regions and stained with hematoxylin-eosin. Multiple drusen and areas of RPE detachment were observed in areas corresponding to the flecks (Figure 4C and D). These drusen were less eosinophilic than typical drusen, but seemed to be otherwise of similar composition, containing C5b-9 complexes and HLA-DR (data not shown). In addition, rare pigment epithelial detachments overlying basal laminar deposits and patches of atrophic RPE were observed, although these were seen less commonly than the peripheral drusen. In the extreme periphery, there was a significant accumulation of basal laminar deposits. In addition, occasional areas of RPE and photoreceptor atrophy were observed overlying an intact Bruch’s membrane in the regions containing flecks ophthalmoscopically.

Immunolocalization of bestrophin was performed on unfixed tissue sections using a monoclonal antibody (NB-300-164). As described previously by Marmorstein et al., bestrophin is primarily localized to the basolateral membrane of the RPE in normal subjects (Figure 4E). Weak labeling was only rarely observed on the apical membrane. However, in this patient with Best disease, intense labeling was apparent along the apical membrane, cytosol, and the basolateral membrane (Figure 4F). Whereas the RPE of these eyes did exhibit considerable lipofuscin, no obvious increase in the size, fluorescence intensity, or number of lipofuscin granules was noted when compared with specimens of age-matched donors.

Best disease is an uncommon condition with a prevalence in Iowa of less than 1 in 10,000. In general, fundus abnormalities associated with Best disease are visible within the first 2 decades of life. Large vitelliform lesions can be seen in individuals as young as 14 months old (E.M.S., unpublished data). However, vitelliform lesions have been documented to develop in a member of a family with classic Best disease as late as 64 years of age. The ophthalmoscopic features of Best disease are typically limited to the macula, although eccentric vitelliform lesions have been described. The presence of midperipheral flecks, as seen in this family, is an uncommon clinical feature of Best disease and might lead to a diagnosis of age-related macular degeneration, pattern dystrophy, or fundus flavimaculatus if additional family members with more typical findings were not available for examination. Optical coherence tomographic findings are similar to those described previously, particularly with the presence of a central optically clear space.

A review of 77 consecutive photofiles of patients with a clinical and molecular diagnosis of Best disease resulted in the identification of 7 patients (9.1%) with small peripheral flecks like those described herein. These patients came from 3 families who all harbored different sequence variations in the bestrophin gene. A histological examination of the flecks in this report revealed clusters of vesicular drusen that were less eosinophilic than typical drusen but were otherwise of similar composition.

In view of the normal localization of bestrophin to the basal aspect of the RPE, it is interesting that the RPE appears histologically healthy in some regions of the macula that exhibit loss of photoreceptors. This finding is in contrast to those of previous studies that demonstrated massive lipofuscin accumulation in the RPE in patients with Best disease. Lipofuscin granules were readily detectable in the RPE with epifluorescence and confocal microscopy, but were not more numerous than expected for a donor of this age. It is possible that the RPE contained more than normal amounts of lipofuscin earlier in life, but that the normal age-related increase in lipofuscin obscured this difference by the ninth decade of life.

In addition to appearing clinically later than typical for Best disease, the histopathologic features of this patient are different than previously described. Attenuation of the outer nuclear layer has been described previously as a finding in Best disease, and the degree of photoreceptor degeneration over a relatively intact RPE layer led some investigators to conclude that the primary lesion in Best disease is in the photoreceptor cells. It is now known that the Best disease gene encodes one member of a family of chloride channel proteins, and that it is expressed by the RPE. The finding that bestrophin is a channel protein that conducts anions through the RPE membrane—and that disruption of the primary sequence of bestrophin has a negative impact on ion conductance—is consistent with the clinically described effect of Best disease on the electro-oculogram. The possible mistargeting of bestrophin, as suggested by immunofluorescence studies, could result in a harmful alteration of the ionic milieu of the subretinal space and contribute to the type of photoreceptor cell loss observed histologically.

In summary, we describe a patient who developed findings of Best disease late in life in association with atypical flecks in the midperiphery of his fundus. Clinico-pathologic correlation identified these flecks as clusters of drusen and regions of basal laminar deposits. In contrast to previously reported histopathologic descriptions of Best disease, the most remarkable changes observed in this subject were a foveal scar and a more widespread loss of photoreceptor cells, which was not confined to the area of the vitelliform lesion. Although it is impossible to assess from a single case, it is possible that some of the damage in those with Best disease who have a Y227N mutation results from the mistargeting of bestrophin.
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